УДК 616.896-053.2-076.5:577.15
https://doi.org/10.20538/1682-0363-2020-4-174-178

# Features of the cytokine profile in children with autism spectrum disorder

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## ABSTRACT

**The aim** of the study was to reveal the particularities of the concentration of cytokines IL4, IL6, IL10, IL17, IFNγ in blood serum in children with autism spectrum disorder (ASD).

**Materials and methods.** The blood samples obtained from children of two study groups: children with autism spectrum disorder ($n$ = 93) and clinically healthy children ($n$ = 30), served as the material for the study. Cytokine concentrations were determined in blood serum using the Bender Medsystems (Austria) kits for IL17A and Vector-Best (Russia) kits for IL4, IL6, IL10, IFNγ. Serum cytokine concentrations were determined by enzyme immunoassay using kits for IL17A (Bender Medsystems, Austria), IL4, IL6, IL10, IFNγ (Vector-Best, Russia). Assessment of cognitive and psychophysiological indicators in children was performed using the Autism Treatment Evaluation Checklist (ATEC).

**Results.** The concentrations of IL17A ($U$ = 54; $p$ = 0.015) and IFNγ ($U$ = 4.64; $p$ = 0.006) were increased and the concentrations of IL6 ($U$ = 327; $p$ = 0.001) and IL4 ($U$ = 177; $p$ = 0.001) were decreased in children with ASD.

The concentration of IL6 correlates with the concentration of IL4 ($r$ = 0.68; $p < 0.05$). The concentration of IL17A correlates with the concentration of IFNγ ($r$ = 0.41; $p < 0.05$), IL6 ($r$ = 0.87; $p < 0.05$) and ATEC score ($r$ = 0.24; $p < 0.05$) in the group of children with ASD.

**Conclusion.** The cytokine imbalance in children with ADS, which was observed in the study, confirms the hypothesis of their participation in the development of the disease and clearly shows the Th17 immunoregulation pathway in the pathogenesis of the autism spectrum disorder.

**Key words:** autism spectrum disorder, cytokines, neuroimmune inflammation, interleukin 17A.

**Conflict of interest.** The authors declare the absence of obvious and potential conflicts of interest related to the publication of this article.

**Source of financing.** The study was sponsored by the Center of Family Medicine (Tomsk, Russia), “Sozidaniye” charity fund (Moscow, Russia), and Association of Parents of Autistic Children “AURA” (Tomsk, Russia).

**Conformity with the principles of ethics.** All parents of the children signed an informed consent for complex research and processing of personal data. The study was approved by the local Ethics Committee at the Center of Family Medicine (Protocol No. 7 of 18.03.2019).

**For citation:** Khudiakova M.I., Cherevko N.A., Novikov P.S. , Berezovskaya K.V. Features of the cytokine profile in children with autism spectrum disorder. *Bulletin of Siberian Medicine.* 2020; 19 (4): 174–178. https://doi.org/10.20538/1682-0363-2020-4-174-178.

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# Особенности цитокинового профиля у детей с расстройством аутистического спектра

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**РЕЗЮМЕ**

**Цель работы:** выявить уровень концентрации цитокинов IL-4, I-L6, IL-10, IL-17, IFNγ в сыворотке крови у детей с расстройством аутистического спектра (РАС).

**Материалы и методы.** Материалом исследования служили образцы крови, полученные от детей двух групп исследования: детей с расстройством аутистического спектра ($n$ = 93) и клинически здоровых детей ($n$ = 30). Средний возраст в обеих группах составил (7 ± 2) лет.

В сыворотке крови методом иммуноферментного анализа определяли концентрацию цитокинов IL-17A (с применением набора Bender Medsystems, Австрия) и IL-4, IL-6, IL-10, IFNγ (Вектор-Бест, Россия). Оценку когнитивных и психофизиологических показателей проводили с помощью анкеты Autism Treatment Evaluation Checklist (ATEC).

**Результаты.** У детей с РАС повышены значения концентрации IL-17A ($U$ = 54; $p$ = 0,015) и IFNγ ($U$ = 4,64; $p$ = 0,006) и снижены – IL-6 ($U$ = 327; $p$ = 0,001) и IL-4 ($U$ = 177; $p$ = 0,001) по сравнению с этими показателями у детей в контрольной группе. Установлены корреляции между концентрацией IL-6 и IL-4 ($r$ = 0,68; $p$ < 0,05); между IL-17A и IFNγ ($r$ = 0,41; $p$ < 0,05), IL-6 ($r$ = 0,87; $p$ < 0,05), количеством баллов ATEC ($r$ = 0,24; $p$ < 0,05) у детей с РАС.

**Заключение.** Установленный нами дисбаланс цитокинов у детей с РАС подтверждает гипотезу его участия в развитии РАС и свидетельствует об Th17-направлении иммунорегуляции в патогенезе расстройств аутистического спектра.

**Ключевые слова:** расстройство аутистического спектра, цитокины, нейроиммунное воспаление, интерлейкин 17А.

**Конфликт интересов.** Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

**Источник финансирования.** Работа выполнена за счет средств ООО «Центр семейной медицины» (г. Томск), благотворительного фонда «Созидание» (г. Москва), с участием ТРОО «АРДА «АУРА» (г. Томск).

**Соответствие принципам этики.** Все родители детей подписали информированное согласие. Исследование одобрено этическим комитетом ООО «Центра семейной медицины» (протокол № 7 от 18.03.2019).

**Для цитирования:** Худякова М.И., Черевко Н.А., Новиков П.С., Березовская К.В. Особенности цитокинового профиля у детей с расстройством аутистического спектра. *Бюллетень сибирской медицины.* 2020; 19 (4): 174–178. https://doi.org/10.20538/1682-0363-2020-4-174-178.

## INTRODUCTION

Autism spectrum disorder (ASD) is a current problem of the 21st century. More new cases of the disease are being revealed every passing year. According to the statistics, the prevalence of ASD is 1:68 among children under 8 years of age [1].

The analysis of scientific data related to ASD over the last 5 years demonstrates the significant growth in global interest in the disease. However, the exact mechanism of ASD pathogenesis remains unknown. One of the modern theories of ASD development is the neuroimmune inflammation hypothesis, which is

associated with food intolerance and cognitive dysfunctions involving innate and acquired immunity and microbiota [2, 3]. In our previous studies, we identified the peculiarities of food hypersensitivity in children with ASD [4].

In this regard, the study of the role of interleukin 17 (IL17) which is thought to be responsible for immune homeostasis control in intestine mucosa, interleukin 6 (IL6), interferon gamma (IFNγ), and anti-inflammatory cytokines such as interleukin 4 (IL4) and interleukin 10 (IL10) in ASD pathogenesis in children is of current interest.

The study aims to reveal the peculiarities of the concentration of cytokines IL4, IL6, IL10, IL17, IFNγ in blood serum in children with an autism spectrum disorder.

## MATERIALS AND METHODS

The study was held in the outpatient department of the Center of Family Medicine (Tomsk, Russia). A total of 123 children selected for the study were split into two groups. The main group was represented by 93 children with a varying severity level of ASD diagnosed 4–5 years ago. 30 somatically healthy children were selected for the control group. The average age of the children in both groups was (7 ± 2) years. Differentiated food hypersensitivity reactions were detected in both groups of children [4].

All parents of the children signed an informed consent for complex research and processing of personal data.

The blood samples taken from antecubital subcutaneous veins in fasting subjects served as the material of the study. Concentrations of IL4, IL6, IL10, IL17, IFNγ were assessed. Cytokines concentrations were determined in blood serum using the Bender Medsystems (Austria) kits for IL17A and Vector-Best (Russia) kits for IL4, IL6, IL10, IFNγ.

To assess the cognitive and psychophysiological changes in children with ADS and to determine the disease severity during the period of blood samples analysis the parents of the children were asked to fill the special questionnaire, the Autism Treatment Evaluation Checklist (ATEC) in accordance with their observations. The scores of the questionnaire of children from the control group were less than 10, confirming the absence of the disease in those children.

The statistical analysis was performed using the IBM SPSS Statistics 23.0 (USA) software. The obtained data were processed using Kolmogorov – Smirnov test, Mann – Whitney U-test, Wilcoxon signed-rank test, and Spearman's rank correlation coefficient. Two-sided $p$-values of $< 0.05$ were considered statistically significant.

## RESULTS AND DISCUSSION

The results of the study listed in the Table 1 show that the IL17A concentration in children with ASD is significantly higher than in the control group ($U = 54$; $p = 0.015$).

Table 1

**Cytokines concentration in children with ADS and children from the control group**

| Cytokines, pg/ml | Children with ADS | | Control group | |
|---|---|---|---|---|
| | Med | IQR | Med | IQR |
| IL4 | 1.75* | 1.00–14.93 | 16.30 | 15.58–17.13 |
| IL6 | 3.20* | 1.00–15.33 | 15.75 | 18.30–20.43 |
| IL10 | 17.45 | 15.38–20.89 | 17.50 | 16.28–19.48 |
| IL17A | 9.58* | 3.76–26.75 | 6.85 | 2.95–15.05 |
| IFNγ | 14.90* | 13.12–16.10 | 13.35 | 11.85–14.20 |

* $p < 0.05$ in children with ADS compared with healthy children; Note. *Med* – median; *IQR* – interquartile range.

IL17A is the pro-inflammatory cytokine produced by several human immune cells such as Th17, neutrophils, peripheral blood mononuclear cells, type 3 innate lymphoid cells (ILC 3). The majority of these cells are situated in barrier tissues where they take part in homeostasis control in the intestine. The cytokines IL17A, IL17F and IL22 produced by ILC 3 cells enhance the barrier function of the intestine by stimulating mucin and antimicrobial peptide production [6]. The disturbance in Th17 cells regulation and IL17A production is associated with the progression of numerous inflammatory and autoimmune diseases including intestinal inflammatory diseases [7].

In literature sources, there are data concerning the IL17A effector role in children's behavioral anomalies caused by maternal immune activation, which shows the relation of neuroinflammatory state and behavioral manifestations [8, 9].

It is important to note that the highest IL17A blood concentrations among the children selected for our study were detected in severe disease manifestations cases determined according to the results of the ATEC test ($r = 0.24$; $p < 0.05$). It may indicate that the increase of IL17A concentration in serum is closely associated with the ADS severity degree.

The IL17 concentration increase in blood serum of children with ADS may be interrelated with the higher levels of IFNγ ($r = 0.41$; $p < 0.05$). According to the present data, the increase in IFNγ production is

associated with the Th17 cell plasticity. The repeated stimulation with various microbial antigens in differentiated cells leads to transcriptional changes in the Th17 line. Examples of such plasticity are Th17/Th1-cells or ILC3 cells which are able to produce both IL17A and IFNγ [10, 11]. In return, IL17A is a stimulator of pro-inflammatory cytokines (such as IFNγ and IL12) produced by macrophages [12]. It may explain the increase of IFNγ concentration in the blood serum of children with ADS comparing with the results in the control group ($U = 4.64$; $p = 0.006$). According to the literature sources, the direct action of IFNγ in high concentrations may interfere with the normal development of the neural system affecting dendrites morphology and synapses formation which can lead to ADS development [13].

According to the results of our study, the IL17A concentration in children with ADS changes in a unidirectional way with the levels of IL6 ($r = 0.87$; $p < 0.05$), concentration of which in children with ADS was decreased ($U = 327$; $p = 0.001$). It is well-known that IL6 is the key factor in Th17 cell differentiation [14]. It is the multi-purpose cytokine which may cause the cell responses to mediating inflammation, neurogenesis, gliogenesis, cell growth, and survival as well as myelinization and demyelination in CNS [15]. Moreover, it has regenerative and anti-inflammatory activity and participates in metabolic and neural processes regulation [16]. Consequently, IL6 takes part in immune system activation, hypothetically causing the development of the ADS-like phenotype in descendants [17].

Whereas, in the group of children with ADS the levels of IL6 correlate with IL4 concentration ($r = 0.68$; $p < 0.05$), which was significantly lower in children with ADS comparing with the control group ($U = 177$; $p = 0.001$). It is proved that IL4 participates in cognitive processes as a neuroprotector [18]. It becomes active during CNS inflammation, causes alternative activation of glia cells, and protects them from apoptosis. Probably, the IL4 concentration decrease is associated with ADS development.

According to the obtained data, there is no significant difference in IL10 concentration in both groups.

## CONCLUSION

The cytokine imbalance in children with ADS, which was observed in our study, confirms the hypothesis of their participation in the development of the disease and clearly shows the Th17 immunoregulation pathway in the pathogenesis of the autism spectrum disorder. However, further studies of the cytokine features in ADS patients are required. It will allow understanding of the mechanism underlying the progression of neurodegenerative diseases, such as ADS, caused by inflammation.

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Received 07.02.2020
Accepted 29.09.2020